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COMPARISON OF URANIUM BIOSORPTION BETWEEN SCYTALIDIUM ACIDOPHILUM AND RHIZOPUS ARRHIZUS

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SUMMARY

Biosorption of uranium from mine biolixiviant and from complex synthetic uranium solutions by the extremely acid-tolerant fungus Scytalidium acidophilum was studied. Uranium uptake at pH 4 was shown to be three times greater than uptake at pH 2.5. Kinetics of biosorption at different cell concentrations were conducted to evaluate the biosorptive capacities of the fungus at pH 4. An uptake of 42 mgU/g was reached within 10 min for a cell concentration of 1.0 g/l. Kinetic studies showed that biosorption is slower and less efficient with mine water compared to synthetic U solution, due to the higher concentrations of competitive ions present in mine water.

Uranium biosorption by S. acidophilum was also evaluated with biosorption isotherms and compared to another fungus, Rhizopus arrhizus. At pH 4, R. arrhizus always showed greater uranium uptake than S. acidophilum for both mine water and complex synthetic solution. Uranium uptake was greater than 37 mgU/g and 51 mgU/g with mine water for S. acidophilum and R. arrhizus respectively, and 65 mgU/g and 122 mgU/g with complex synthetic uranium solution.

KEY WORDS

Uranium biosorption, Scytalidium acidophilum, Rhizopus arrhizus, Process and mine water, Uranyl solution.

INTRODUCTION

Biomass may be extensively used in the next few years as a means to remove and concentrate uranium from process solutions or mine waters containing low concentrations of uranium. Many microorganisms have been tested for U adsorption over the past ten years. Initially, all biosorption studies were conducted using uranyl nitrate or uranyl sulphate solutions. Screenings of microorganisms were carried out and comparative studies were made (1-8). Among all the microorganisms tested (bacteria, yeasts, algae and fungi), one fungus, Rhizopus arrhizus, was found to be the most effective uranium biosorbent (2). Several authors have studied, in detail, the mechanism of uranium biosorption by this fungus (9-11). Other studies were carried out on more complex natural solutions. The effect of anions and cations on uranium uptake capacity by R. arrhizus has been investigated (12-14). Finally, uranium biosorption by R. arrhizus and other microorganisms from process solutions has been reported (15-18).

Uranium process solutions have low pH values, often lower than 2.5. However, the pH must be between 4 and 5 for maximal uranium biosorption by R. arrhizus. At higher pH values U may precipitate which would result in some uranium being lost. To solve this problem, it would be advantageous to find a microorganism with high uranium uptake capacity at low pH. This study was carried out to assess the biosorption of uranium by the fungus, Scytalidium acidophilum, which tolerates extreme acidity and high salt concentrations (19,20). Both complex synthetic uranium solutions and uranium process mine water were used and a comparison of the uranium biosorption capacity under the same conditions was made between S. acidophilum and R. arrhizus.

MATERIALS AND METHODS

Preparation of biomass

The microorganisms used in this study were Scytalidium acidophilum UAMH 3721 (University of Alberta Mold Herbarium) and Rhizopus arrhizus ATCC 6204 (American Type Culture Collection). For preliminary kinetics experiments, S. acidophilum was cultivated in 100 ml of Nutrient Broth (Difco). The medium was adjusted to pH 2.0 with sterile H_2SO_4 as Gould et al (19) reported that growth of S. acidophilum was optimal at this pH. Ten ml of a 7-day culture was used to inoculate 6 250 ml-flasks containing 100 ml of Potato Dextrose Broth (Difco) at pH 2.0. The cultures were incubated in a New Brunswick Scientific Gyrotary G-25 Incubator at 200 rpm and 27°C for 5 days. These cultures were subsequently used to inoculate 1-liter cultures of the same medium. After 4 days of incubation at 27°C, biomass was harvested by vacuum filtration through glass fiber filters (Whatman No1 Qualitative) and washed with distilled water at pH 2.0. Biomass was not dried but was stored at 4°C.

For comparative experiments, both S. acidophilum and R. arrhizus were cultivated in Potato Dextrose Broth (Difco). These cultures were used to inoculate 3, 1-liter flasks of Potato Dextrose Broth (Difco) adjusted at pH 2.0 for S. acidophilum and pH 4.5 for R. arrhizus, and incubated at 200 rpm and at 27°C for 5 days. Biomass of both fungi was filtered and washed repeatedly with distilled water at pH 2.0 for S. acidophilum and pH 4.5 for R. arrhizus. The biomass was dried at 65°C for 24 h and ground in a marble

mortar and pestle to produce particles of less than 300 μm. Biomass was stored at 4°C, prior to use.

Uranium analysis and calculation

Uranium analyses were by fluorimetry using the fused bed technique (Model 26-000 Jarrell-Ash Fluorimeter). A fused U-NaF bed was exposed to ultraviolet light and the resulting fluorescent emission was measured by a photomultiplier tube. Repeated analyses indicated that the error of this technique was ± 2 to 5 %.

For each experiment, the initial concentration of uranium was measured at least three times and the average value was used for all calculations. The uranium concentrations of all liquid samples and biomass wash water were determined as well. Every sample of dry biomass was digested as follows: biomass samples and filters were placed in 10 ml of distilled water in a 300 ml beaker. Bromine (2 ml) was added to biomass samples and heated until dryness. Then 250 ml of Aqua Regia (HNO_3:HCl, 1:1) was added and boiled until the biomass was completely oxidized. The resulting liquid was filtered through a 0.45 μm cellulose acetate membrane filter (Millipore) and the filtrate was analyzed for uranium.

The uranium uptake was calculated by mass balance from the analyses of the liquid samples and the biomass samples.

Uranium Solutions

Most of the experiments in this study were carried out with a synthetic solution of uranium, the composition of which was similar to that of Denison Mine water described by McCready et al (21). Solutions were freshly prepared for each experiment with the following composition: $UO_2(NO_3)_2.6H_2O$, $MgSO_4,7H_2O$, $Fe_2(SO_4)_3,nH_2O$, $NaNO_3$, $(NH_4)_2SO_4$ and KH_2PO_4. Before filtration, the composition of the synthetic solution was : $[H_2PO_4^-]$=15 mg/l, $[Mg^{2+}]$=11 mg/l, $[NH_4^+]$=45 mg/l, $[NO_3^-]$=190 mg/l, $[SO_4^{2-}]$=206 mg/l, [U(VI)]=250 mg/l and $[Fe^{3+}]$=50 mg/l.

The iron concentration was much lower than that of Denison Mine water in order to minimize the volume of the precipitate produced at a pH greater than 3 and thus reduce the loss of uranium.

Comparative kinetics experiments and biosorption isotherms were made with a biolixiviant from Denison Mines bacterial leaching stopes.

Kinetics of Uranium Uptake

To study the effect of pH and biomass/liquid ratio, a known weight of wet biomass was added to 600 to 1000 ml of uranium solution. Biomass and solution were continuously agitated using a magnetic stirrer. All experiments were conducted at 22-24°C.

At predetermined intervals a sample of 90 to 100 ml was taken. The sample was filtered on a tared 0.45 μm Millipore filter and an aliquot of the filtrate was analyzed for uranium. The biomass was either dried for 36 h at 25°C for the pH study or washed with 100 ml of distilled water at pH 4.0 and

dried under the same conditions for the biomass/liquid ratio study. After vacuum filtration an aliquot of wash water was taken for uranium analysis. During the course of these experiments, pH was continuously monitored and maintained at the required value.

Large samples were used in this study to reduce the % error on analysis. If small samples (1-5 ml) were collected, the % error increased markedly due to evaporation of liquid samples prior to analysis or due to the low concentration of uranium. Biomass/liquid ratios were calculated versus time and were shown to be constant with time using this sampling technique.

The same sampling technique was used for comparative kinetic studies and each sample of biomass was washed with 100 ml of distilled water at pH 4.0. For these studies, dried ground biomass was utilized, therefore the biomass was recovered using Whatman filters (No1 Qualitative) as the biomass had a large particle size.

Biosorption Isotherms

For both Rhizopus arrhizus and Scytalidium acidophilum two isotherms were determined: one with synthetic uranium solution and the other with mine process water. For the first set, a 1000 mgU/l solution was prepared. $H_2PO_4^-$, Mg^{2+}, NH_4^+, NO_3^- and Fe^{3+} concentrations were the same as described earlier and pH was adjusted to 4.0 with NaOH. After filtration several uranium solutions were prepared by dilution of the initial solution with a pH 4.0 mineral salts solution without uranium. Ground dried biomass (0.150 g) was added to 100 ml of uranium solution and placed in an incubator shaker for 5 days at 25°C and 150 rpm. pH was measured initially and 5 days

later prior to filtering the solution. Biomass was recovered on a 0.45 µm Millipore filter, washed with 100 ml of distilled water, dried for 24 h at 65°C and weighed.

For the second set, mine process water was adjusted to pH 4.0 and filtered through 0.45 µm filters. A series of experiments were conducted in which the dry cell weight was varied; the analytical procedure for this series was, however, as described above.

RESULTS

pH effect

The effect of pH on uranium biosorption by Scytalidium acidophilum was studied. Uranium uptake kinetics were determined for pH values between 2.0 and 4.0 with living biomass. This pH range was chosen because the mine process water pH is generally about 2.5 and because uranium biosorption from iron rich mine water at a pH above 4 or 4.5 can lead to loss of uranium in the iron hydroxide precipitate.

Uranium uptake (mgU/g dry biomass) was calculated by mass balance from solution analysis. The error on the uptake value varies from 5 to 10 %. It can be concluded from these results that uranium biosorption is significantly higher at pH 4.0 than at pH 3.5 or 2.5; one hour uranium uptake is 23 mgU/g at pH 4.0 and only 7 mgU/g at pH 2.5 (Fig. 1). Moreover uranium uptake is a function of pH in the range 3.5 - 4.0. The biomass/liquid ratio was 6.5 ± 0.3 g/l for all the pH values.

Kinetics of uranium biosorption by S. acidophilum was rapid and significant biosorption was observed after 20 or 30 seconds in the pH-range studied.

Effect of Biomass/Liquid Ratio (R)

Adsorption kinetics were assessed at pH 4.0 with synthetic U solution for different biomass/liquid ratios. Figure 2 presents four curves for various biomass/liquid ratios ranging from 1.0 to 6.2 g dry cells/l. Each uptake value on the graph is the average between the uptake calculated by mass balance from solution analysis and the uptake measured directly on biomass after chemical oxidation. From Figure 2, the lower the biomass/liquid ratio, the less variance in uranium uptake with time. As expected, the uranium uptake increased when the biomass/liquid ratio (R) decreased. After 60 minutes of contact, the uranium uptake was 22 ± 2 mgU/g for R=6.2 g/l and 45 ± 7 mgU/g for R=1.0 g/l. There is no significant difference between R=1.7 g/l and R=3.2 g/l. In contrast, uranium adsorption was highest (55%) for the highest cell concentration and lowest (20%) for the lowest cell concentration tested.

Comparison of S. acidophilum with R. arrhizus

Kinetics studies and biosorption isotherms were determined with both S. acidophilum and R. arrhizus. Uranium biosorption was studied with an synthetic uranyl solution and Denison Mine water. For kinetic experiments,

the initial uranium concentration of the synthetic solution after filtration was 215 mg/l and the mine process water was 102 mg/l.

Comparative kinetics are presented in Figures 3 and 4 in which the uptake was calculated by solution mass balance. The values measured following biomass oxidation compared favorably with those obtained from the solution mass balance calculation. For both synthetic solution and mine process water, R. arrhizus showed a greater U uptake than S. acidophilum. After 90 minutes, with synthetic solution, R. arrhizus took up 80 mg/g, which was twice the S. acidophilum uptake. In the case of mine process water, the difference was less (R. arrhizus: 26 mg/g and S. acidophilum: 17 mg/g at 150 min). Uranium % adsorption versus time is plotted in Figures 5 and 6. High % adsorption was obtained with R. arrhizus: >75% at 150 min compared to 40% with S. acidophilum. Both fungi exhibit distinctly different adsorption kinetic rates between the synthetic U solution and the mine process water. All experiments were conducted with similar biomass/liquid ratios; thus it is apparent that uranium adsorption was slower with the mine water than that with the synthetic U solution. Also, the curve for S. acidophilum indicates that uranium uptake by living biomass was slightly less than uranium uptake of biomass dried at 65^{o}C.

Comparative biosorption isotherms are presented in Figures 7 and 8. In these experiments pH was not kept constant. All experiments were initiated at pH 4.0; as adsorption proceeded, the pH decreased (Table 1). These results confirm the findings of the kinetic study. With synthetic solution, the maximum uranium uptake was 122 mgU/g and 65 mgU/g for R. arrhizus and S. acidophilum respectively. The difference in U uptake between the two fungi was less significant with mine water: >51 mgU/g for R. arrhizus and >37 mgU/g

for S. acidophilum. Using mine process solution, the biosorption isotherm of S. acidophilum was linear whereas it was logarithmic for R. arrhizus. Uranium uptake from mine process water was twice as high at equilibrium (Fig. 8) when compared to the uptake observed in 150 minutes contact (Fig. 4), indicating that U adsorption is very slow with the mine process solution.

DISCUSSION

Several microorganisms were reported to achieve a high uranium uptake with a synthetic U solution (2,3). Among these microorganisms, R. arrhizus exhibited uranium uptakes greater than 180 mgU/g at pH 4. Uranium biosorption was reported to be affected by different parameters such as the pH and the concentrations of competing ions present in the U solution. Uranium uptake at pH 4 was two to three times greater than at pH 2 (2,12). Two distinct groups of biomass were identified: biomass in which pH did not affect uranium uptake between pH 2 and pH 4; and biomass that was affected by pH in a manner similar to R. arrhizus. Other studies showed the same effect of pH on biosorption of uranium from mine process solutions (15-17). The optimum pH for uranium biosorption was often found to be between 4 and 5 for fungi. Uranium biosorption by R. arrhizus was considerably reduced in the presence of anions (13) or cations such as Fe^{2+} or Zn^{2+} (9,14). Thus, ferric ions were reported to be the most effective competitors of UO_2^{2+} in Denison Mine water (17). It was also shown that sulphate complexes present in these process solutions were not adsorbed by R. arrhizus. All the microorganisms tested, to date, exhibited less U uptake from mine process liquors than from synthetic U solutions. Uranium uptake by R. arrhizus with mine process liquor

was found to be 32 mgU/g in one hour at pH 5 (17) or 10-15 mgU/g at pH 4 (15). An earlier study reported uptake values of 42 mgU/g at pH 3.5 with a process solution with an initial U concentration of 200 mgU/l (16). A Penicillium was reported to adsorb 150 mgU/g from mine process liquor (18).

The biosorption mechanisms were also investigated. It is now well known that during uranium biosorption by R. arrhizus, there is a competition for uptake sites between H_3O^+ and UO_2^{2+} (2,9,10,11). Several authors studied the correlation at the equilibrium between the H^+ ion concentration in solution and the uranium uptake for different microorganisms. It was shown that during a continuous experiment with R. arrhizus, two moles of H^+ ions were exchanged for one mole of uranyl ions (11). Another study with Streptomyces longwoodensis showed that during uranium biosorption, H^+ ions were displaced by uranyl ions in a simple stoichiometric ratio of 1 (3). Yakubu explained uranium biosorption by the fungus Aspergillus niger as simple ion exchange between H^+ and UO_2^{2+} (6). In addition to this mechanism, the negatively charged cell wall may be involved due to carboxylate groups present in the cell wall. Tsezos showed with infra-red studies the coordination of uranium with nitrogen in the cell wall chitin of R. arrhizus. The maximum level of uranium that chitin could accumulate was 10 mgU/g at pH 4 (10).

In a previous study, kinetics showed that the uranium uptake by two species of Actinomycetes was very rapid and reached a plateau after 2 minutes of contact. Uranium biosorption from uranyl nitrate solution was very rapid for most of the microorganisms including Pseudomonas aeruginosa (<10 s) (4). Using R. arrhizus, an initial equilibrium plateau was reached within the first 60 s of contact.

The effect of biomass concentration has been studied earlier with many microorganisms (1). For most of them, uranium uptake decreased when the cell concentration increased.

Comparison of the data from the current study to that of previous studies is difficult due to the variations in experimental design. The results of the pH study suggest that the cell wall and the chitin may have the same role in uranium biosorption by S. acidophilum as in U biosorption by R. arrhizus. As with R. arrhizus, chitin alone cannot explain a high uranium uptake (>65 mgU/g). Thus, several mechanisms may be involved in uranium biosorption by S. acidophilum, including ion exchange between H^+ and UO_2^{2+}. The results of the cell wall concentration study were in good agreement with those reported previously. With S. acidophilum a plateau was reached within about the first two minutes at high R values. However, at lower R values it seems that the equilibrium was reached more slowly, probably due to the lower probability of collision between cell wall active sites and the uranyl ions in solution. Equilibrium was a function of the ratio of biomass to solution volume as well as time.

Uranium uptake for both R. arrhizus and S. acidophilum were always less with mine process solutions than with synthetic U solutions. These results are in agreement with studies reported previously (15-18) and suggest that the difference in U uptake between synthetic solutions and process solutions depends on the nature of the uranium compounds in solution and not on the cell structure. During uranium biosorption by R. arrhizus from mine process water, an equilibrium plateau was reached with R. arrhizus and mine process water in one hour but no plateau was reached using S. acidophilum after 150 minutes contact. These results suggest that the mechanism of UO_2^{2+}

adsorption by R. arrhizus is different from that of S. acidophilum, although this difference is not evident with synthetic solutions.

In addition, the results of the biosorption isotherms suggest that the mechanism of UO_2^{2+} adsorption by S. acidophilum may not be the same with synthetic solutions as with mine process water as the isotherms are quite different. The adsorption isotherms of R. arrhizus are Freundlich-type isotherms. However, the biosorption isotherm of S. acidophilum with synthetic solutions is not adequately described by this type of isotherm. In the case of process mine water, the uranium uptake by S. acidophilum is linearly proportional to the equilibrium concentration, that is the uranium concentration in solution is not a limiting factor for the uranium uptake, and uranium uptake could be greater if the initial concentration of uranium in the mine water was higher. This isotherm is characteristic of a Langmuir-type isotherm.

In conclusion, S. acidophilum has a lower uranium uptake capacity than R. arrhizus. With mine process water, the difference is less obvious than with a synthetic uranium solution. S. acidophilum may be competitive in adsorptive capacity to R. arrhizus if mine process waters containing a higher concentration of uranium were tested.

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Figure 1: pH influence on kinetics of uranium uptake by *S. acidophilum*. Synthetic solution: C_o=245 ± 0.5 mgU/l. R=6.5 ± 0.3 g/l.

Figure 2: Biomass/solution ratio (R) influence on kinetics of uranium uptake by *S. acidophilum*. Synthetic solution: C_o=250 ± 10 mgU/l. pH=4.0.

Figure 3: Comparative kinetics of uranium uptake from synthetic solution. C_o=215 mgU/l. R=2.3 g/l. pH=4.0.

Figure 4: Comparative kinetics of uranium uptake from mine process water. C_o=102 mgU/l. R=2.3 g/l. pH=4.0.

Figure 5: Comparative kinetics of uranium recovery from synthetic solution. C_o=215 mgU/l. R=2.3 g/l. pH=4.0.

Figure 6: Comparative kinetics of uranium recovery from mine process water. C_o=102 mgU/l. R=2.3 g/l. pH=4.0.

Figure 7: Comparative biosorption isotherms with synthetic solutions. C_o=40-500mgU/l. Initial pH=4.0. T=25°C.

Figure 8: Comparative biosorption isotherms with mine process water. C_o=115 mgU/l. Initial pH=4.0. T=25°C.

TABLE 1

Final pH values and uranium uptakes (mgU/g) at equilibrium (5 days). Initial pH=4.0 ± 0.05

Mine water C_o=115 mgU/l				Synthetic solution			
R. arrhizus		S. acidophilum		R. arrhizus		S. acidophilum	
mgU/g	pH	mgU/g	pH	mgU/g	pH	mgU/g	pH
51.4	3.66	37.4	3.51	31.3	4.56	65.3	3.44
46.7	3.72	27.1	3.57	26.7	3.53	82.6	3.44
39.3	3.53	32.7	3.52	60.0	3.48	107.0	3.84
30.3	3.53	21.8	3.35	63.0	3.49	121.0	3.53
24.9	3.57	19.2	3.27	64.7	3.49	122.0	3.52
		18.7	3.23				

Figure 1

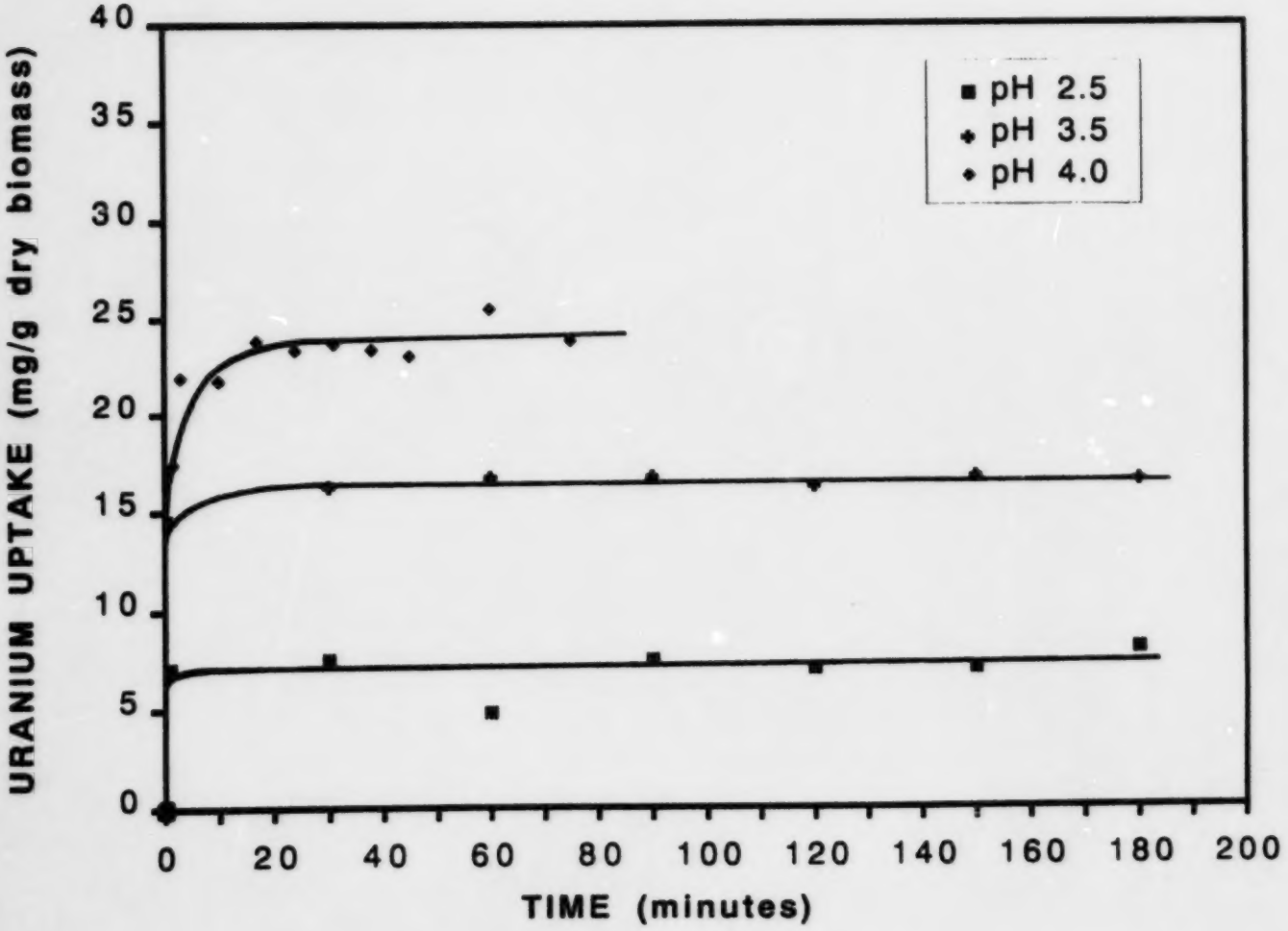
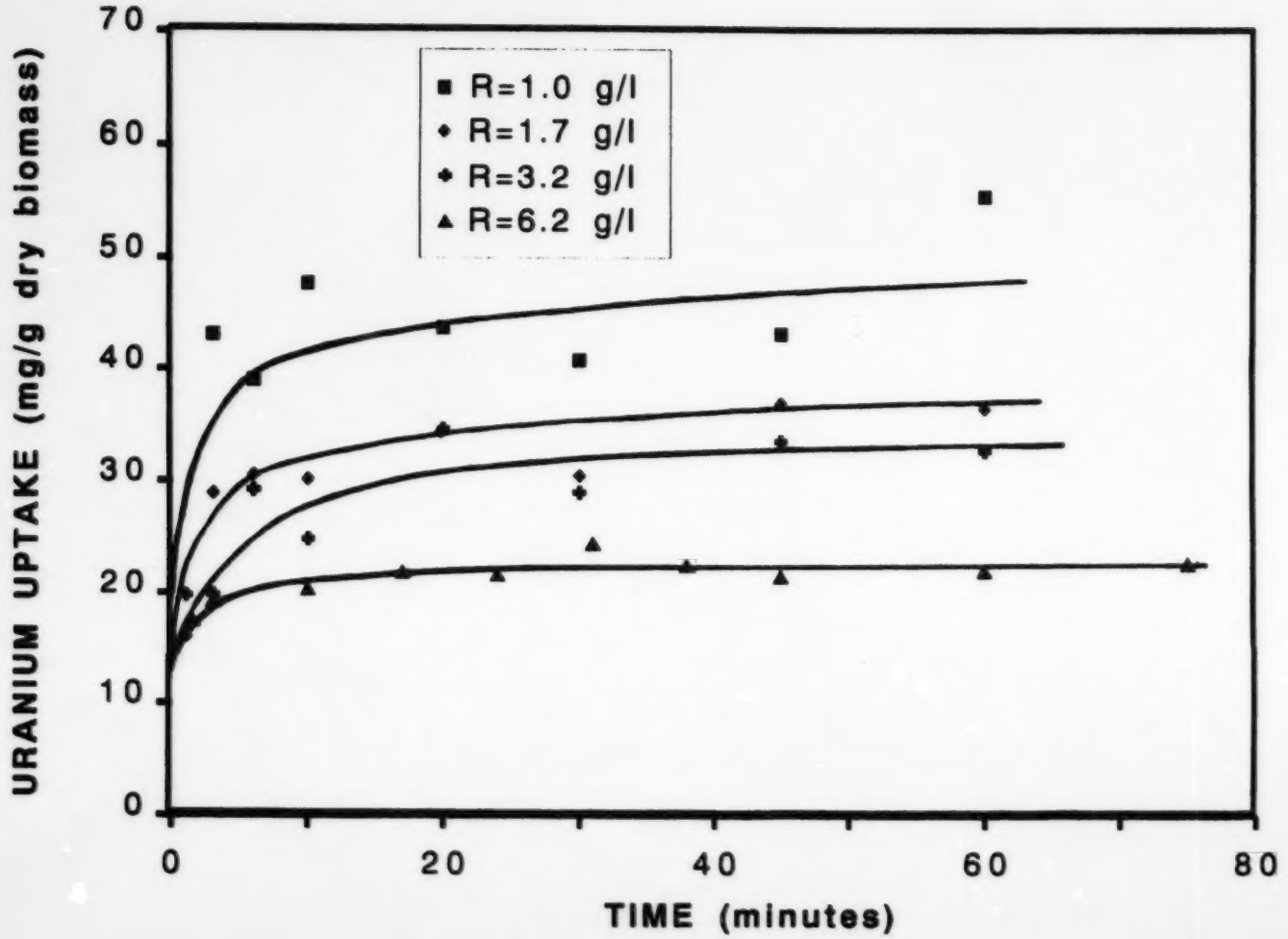

URANIUM UPTAKE (mg/g dry biomass)
TIME (minutes)
pH 2.5
pH 3.5
pH 4.0
0
5
10
15
20
25
30
35
40
0
20
40
60
80
100
120
140
160
180
200

Figure 2


R=1.0 g/l
R=1.7 g/l
R=3.2 g/l
R=6.2 g/l
URANIUM UPTAKE (mg/g dry biomass)
TIME (minutes)
0
10
20
30
40
50
60
70
0
20
40
60
80

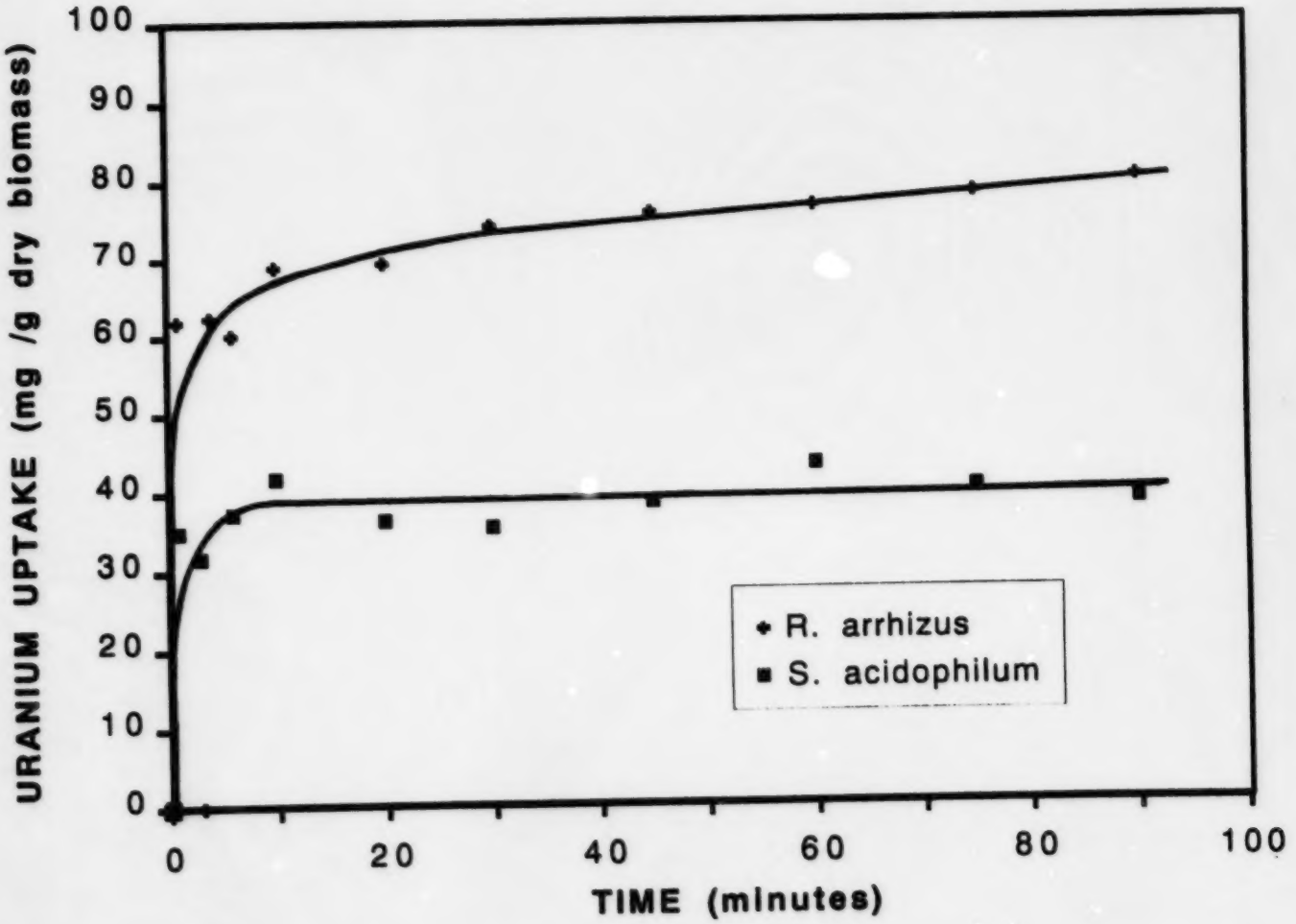

URANIUM UPTAKE (mg /g dry biomass)
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
TIME (minutes)
R. arrhizus
S. acidophilum

Figure 4

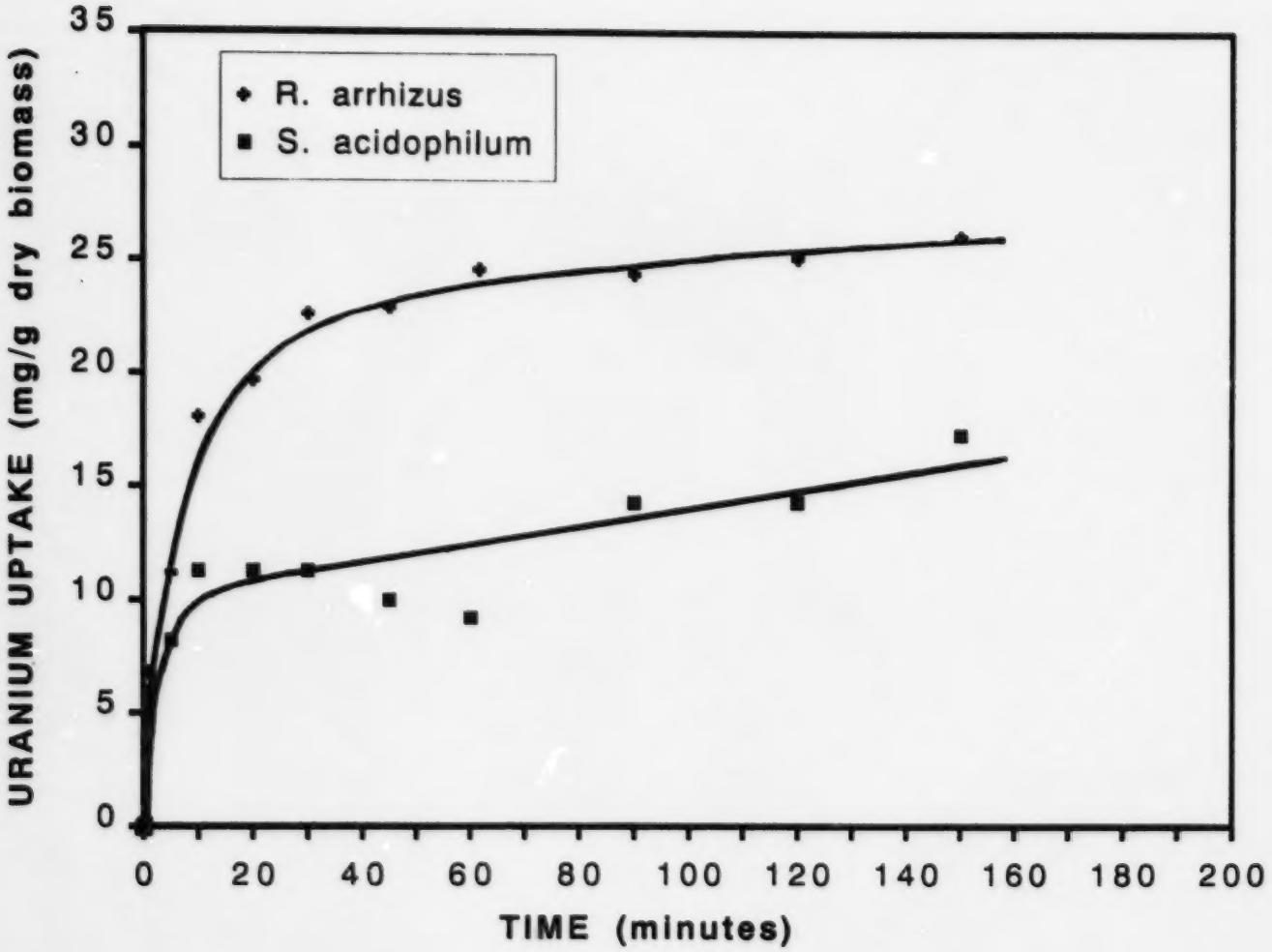
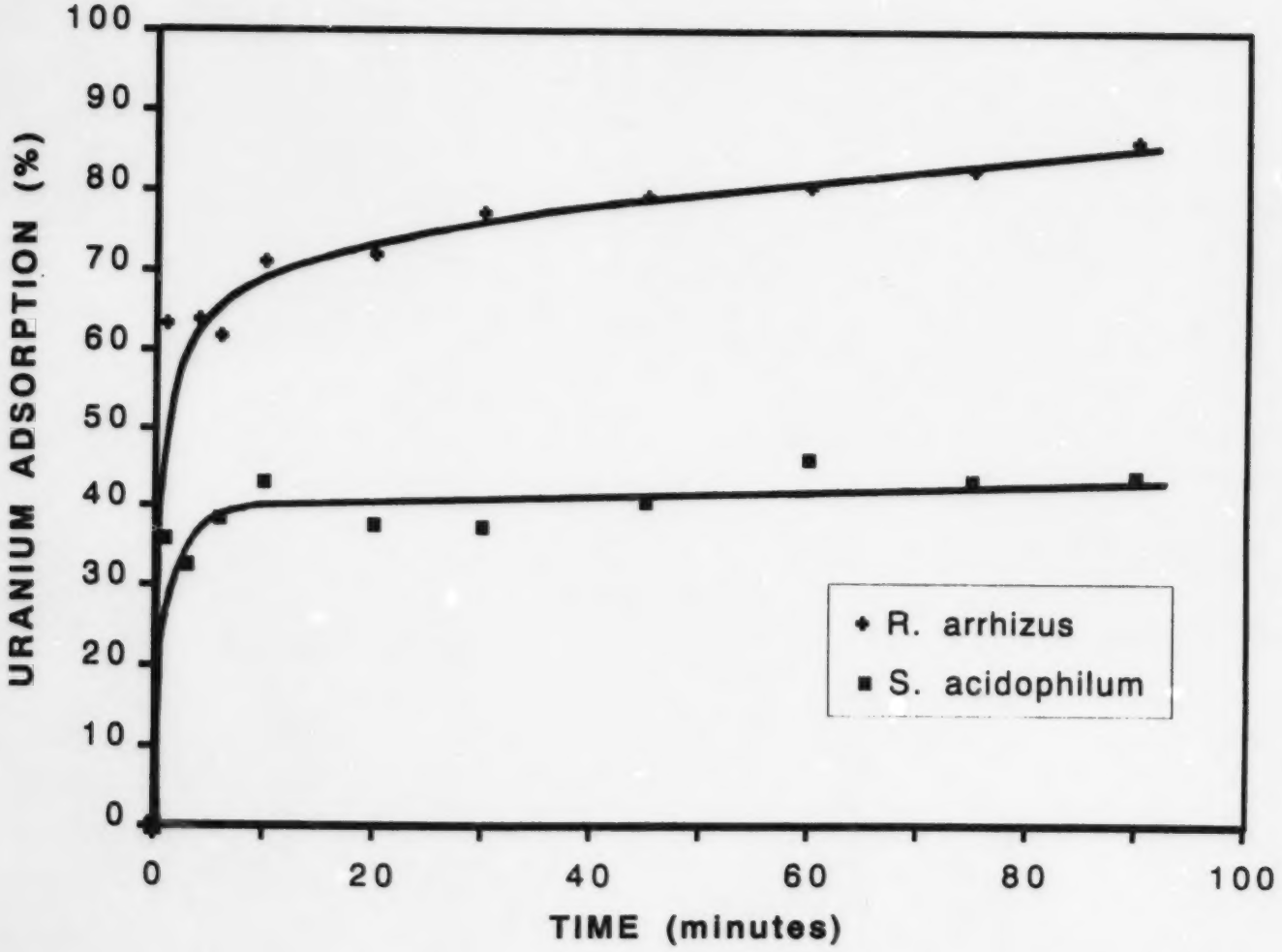
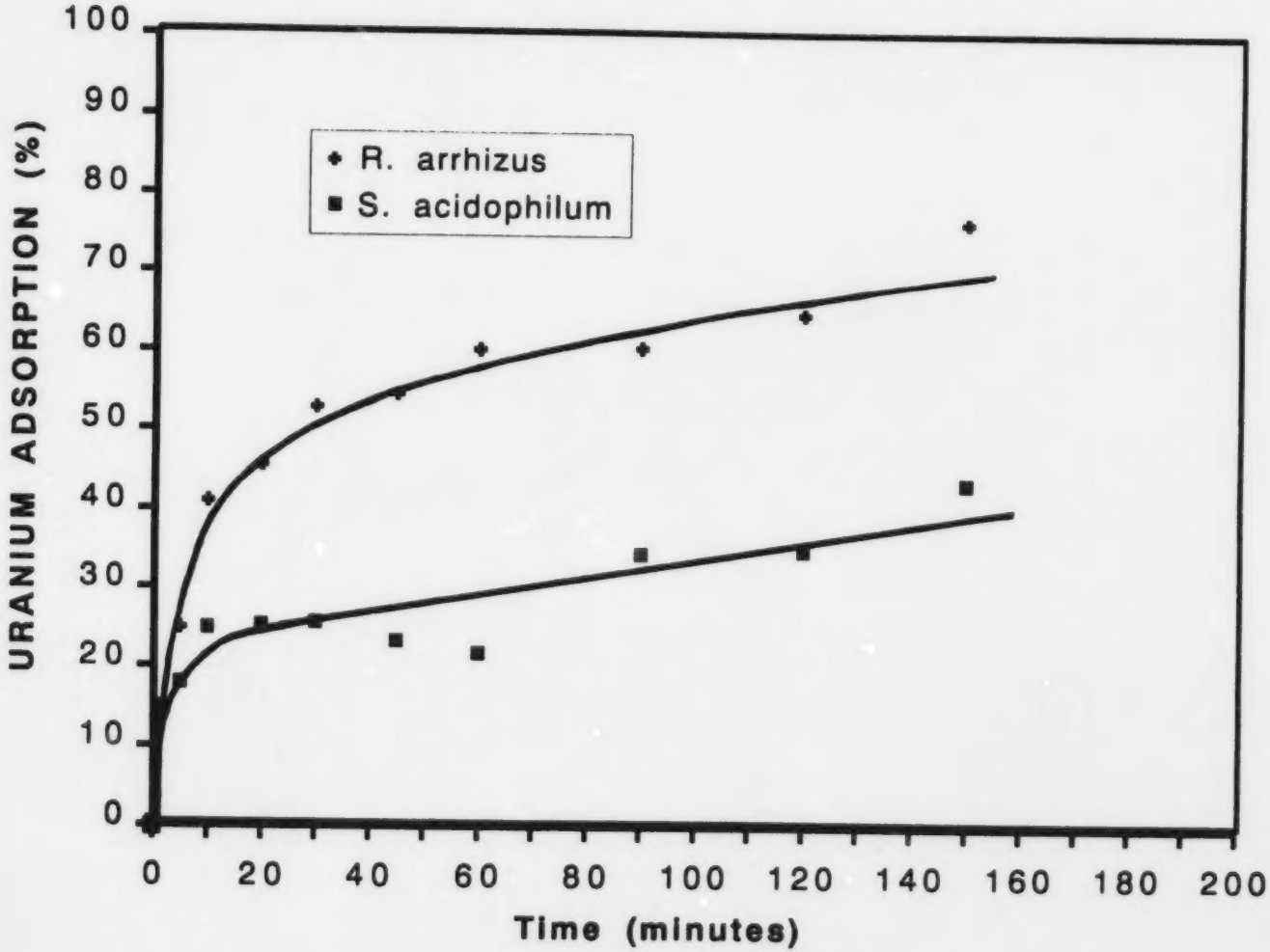

R. arrhizus
S. acidophilum
URANIUM UPTAKE (mg/g dry biomass)
TIME (minutes)
0
5
10
15
20
25
30
35
0
20
40
60
80
100
120
140
160
180
200

Figure 5


URANIUM ADSORPTION (%)
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
TIME (minutes)
R. arrhizus
S. acidophilum


URANIUM ADSORPTION (%)
100
90
80
70
60
50
40
30
20
10
0
R. arrhizus
S. acidophilum
0
20
40
60
80
100
120
140
160
180
200
Time (minutes)

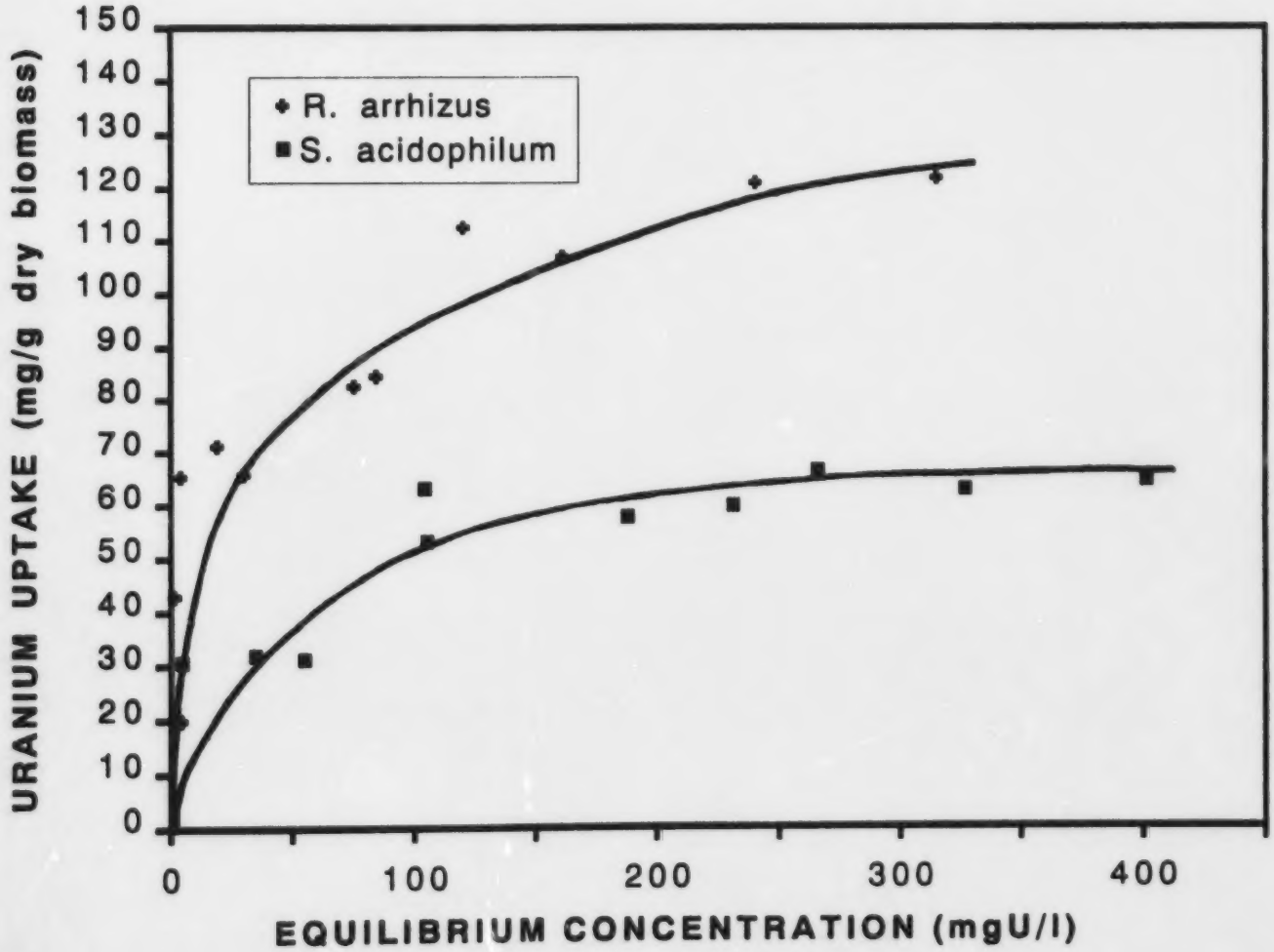
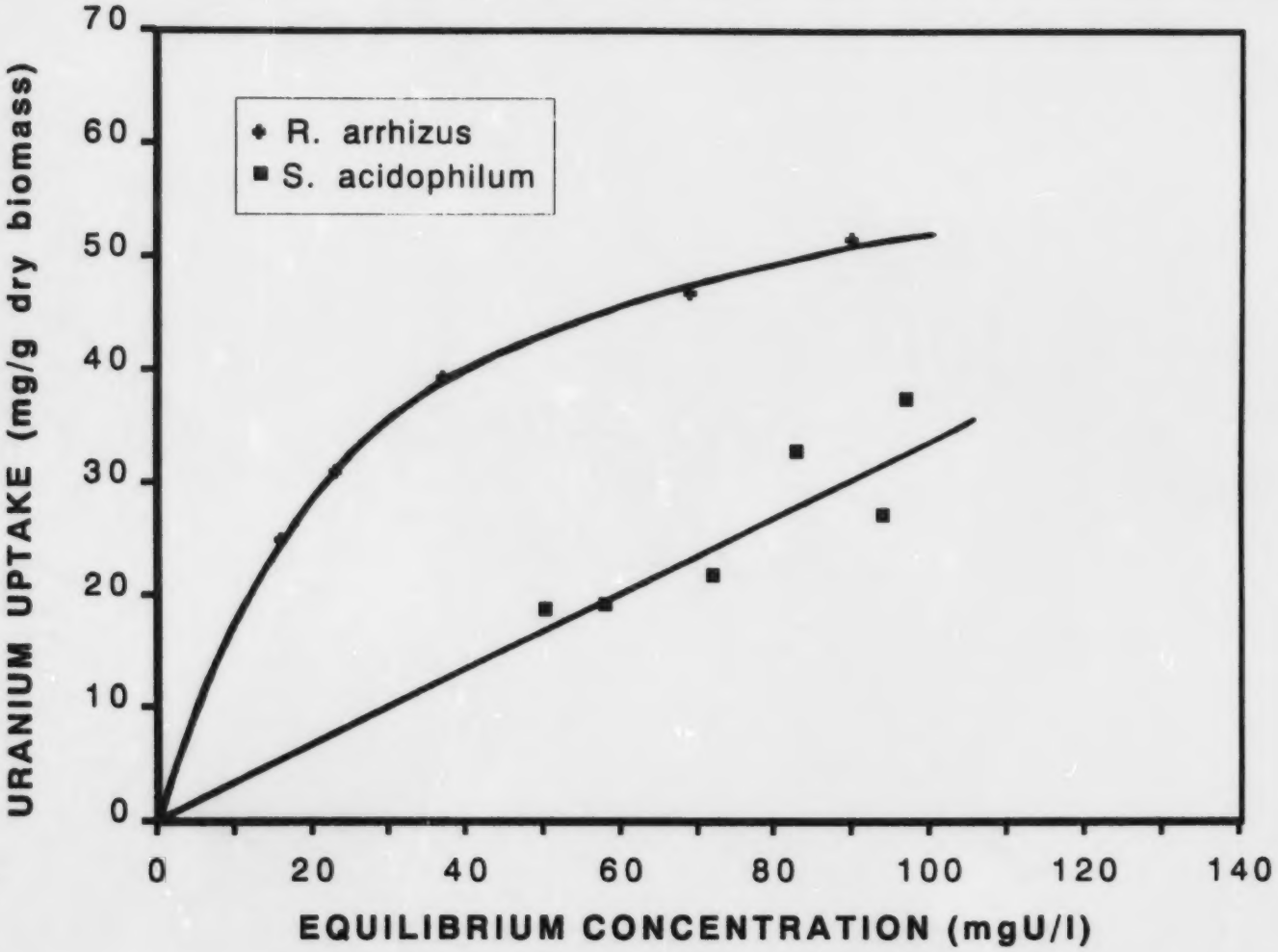

R. arrhizus
S. acidophilum
URANIUM UPTAKE (mg/g dry biomass)
EQUILIBRIUM CONCENTRATION (mgU/l)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
0
100
200
300
400

Fig. 8


R. arrhizus
S. acidophilum
URANIUM UPTAKE (mg/g dry biomass)
EQUILIBRIUM CONCENTRATION (mgU/l)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
140